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Root Uptake and Xylem Translocation of Pesticides from Different Chemical Classes

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Abstract: A pressure-chamber technique was used to study the root uptake and xylem translocation of some fungicides, herbicides and an insecticide from different chemical classes in detopped soybean roots. Physiological parameters such as K^+ leakage from roots, K^+ concentrations in the xylem sap, and protein and ATP levels in the root cells were measured so as to evaluate any potential damage of this technique to the root system. HPLC was used to quantify the compounds in the xylem sap. The pressure-chamber technique has proved useful to study the root uptake and translocation of pesticides, does not damage the root system, and allows one to obtain appreciable volumes of xylem sap that can be analysed directly by HPLC, thus avoiding dependence on the availability of radio-labelled compounds. The concentration of each pesticide in the xylem sap showed a steady-state kinetic profile. Non-linear regression analysis was used to calculate the steady-state concentration and the time required to achieve 50% of the steady-state concentration ($TSSC_{50}$). $TSSC_{50}$ was well correlated with log K_{ow}; the more lipophilic the compound the more time was required to reach the steady-state concentration. The efficiency of translocation was assessed by the transpiration stream concentration factor (TSCF) and a non-linear relationship between TSCF and log K_{ow} was observed. The highest TSCF values were measured for those compounds with log K_{ow} values around 3, a lipophilicity value similar to that reported earlier in an analogous experiment with detopped soybean plants but slightly higher than that reported in earlier experiments with intact barley plants. Lower TSCF values were obtained with chemicals with log K_{ow} values below as well as above 3.

Key words: pressure-chamber, pesticide translocation, TSCF, $TSSC_{50}$, log K_{ow}

1 INTRODUCTION

Pesticides able to enter plants and to be transported in the vascular system are defined as systematic. Systemic distribution in plants can be achieved following foliar application as well as by uptake via roots. Both phloem and xylem translocation of agricultural chemicals are active research areas, but root-to-shoot translocation via xylem is less studied because of the technical difficulties associated with root experiments.

Although it is well known that some pesticides can enter plants following root uptake,[1–5] only in the last ten years have theories been developed that explain the rationale of root uptake and translocation. Root uptake plays a significant role not only for soil-applied pesticides but also for foliar ones, because these may escape from the target sites, reach the soil and then be taken up by roots. There is evidence that some pesticides that are usually applied to shoots can also become systemic when applied to roots.[6]

A systemic pesticide can be adsorbed by roots via either the liquid or the vapour phase in the soil, the proportion taken up by each route depending largely on the physicochemical properties of the compound, as well-described by the Henry's law constant.[7] Roots usually take up water most rapidly in the region 10–

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100 mm behind their tips[8,9] and pesticides should be taken up in the same way.[5] Since some pesticides are lipophilic, their uptake could remain substantial even in regions of roots known to be of low permeability to water. These arguments are intriguing and should be taken into account when dealing with root uptake and transport of pesticides.

A solute that is taken up by roots from the soil solution can take, alternatively or simultaneously, two pathways to reach the xylem vessels along which it is transported via the transpiration stream: (a) the apoplastic pathway, via the cell wall space of the epidermis and cortex and across cell membranes at the endodermis region;[7] in those species that develop an exodermal Casparian strip,[10] the solute must also cross the membrane at the exodermis region; (b) the symplastic route, crossing cell membranes of root hairs, epidermis or cortex and moving to stele by plasmodesmata and/or by membrane permeation. It is the balance between the pesticide distribution in the apoplastic-symplastic compartments that determines the overall transport pattern.[7] One of the crucial factors in transport is thus the rate of passage of compounds across the membranes that act as barriers to flow. Less-lipophilic pesticides take the apoplastic pathway before reaching the endodermis, while more-lipophilic pesticides tend to cross membranes and be partitioned into lipophilic tissue along the pathway.[11] Among the physicochemical properties necessary for chemical transport following uptake by roots, lipophilicity and acid strength play an important role.[12–17]

Among the techniques used to study the movement of pesticides in plants following root uptake is that in which radiolabelled pesticide is applied to the plant roots in nutrient solution and the amount accumulated in different parts of the plant measured.[18] The use of cold compound in translocation tests is often necessary because few pesticides are available in radiolabelled form during the early stages of screening for activity. Unfortunately the extraction and clean-up procedures are time-consuming and may give erratic results when working with the concentrations normally present in soil or solution. These drawbacks can be overcome by measuring pesticide concentration directly in xylem sap. Concentrations of radiolabelled xenobiotics have been measured in xylem sap collected from stem or leaf bases using a vacuum tube[19] or by a pressure-chamber device which gives a realistic flow rate through stem segments.[20–22] The pressure-chamber technique, widely used to study the hydraulic and osmotic properties of root systems,[23–27] could be a useful tool to obtain a considerable volume of xylem sap which can then be analysed by chromatographic techniques. The adaptation of this technique for root-to-shoot translocation of non-radiolabelled oxabicycloalkane compounds in detopped soybean roots has been reported[11] with quantification achieved by GC/MSD. Bromilow *et al.*[28] collected phloem sap directly from *Ricinus communis* L. that was previously injected with solution containing non-radiolabelled compound and analysed the extracts by HPLC.

This paper describes the application of a pressure-chamber technique to study the root-to-shoot translocation in soybean of non-radiolabelled pesticides. The aim of this work was to extend the current theories on uptake and xylem loading to pesticides belonging to different chemical classes. HPLC was used to measure the amount of the active ingredients directly in the xylem sap, an approach that seems attractive for the testing of cold compounds. Some physiological parameters of the soybean plants were also measured in order to check the suitability of this technique as a valid test in translocation study.

2 MATERIAL AND METHODS

2.1 Plant materials

Soybean (*Glycine max*. L., cv. Adel) seeds were germinated in the dark at 26°C on paper towelling saturated with water. Four to five days later, seeds with radicles 1–2 cm long were transferred to a beaker containing aqueous calcium sulfate (0·5 mM). The seedlings were supported so that only the radicles dipped into the solution, which was aerated continuously. The seedlings were kept for three days in the dark to stimulate internode elongation so as to obtain seedlings with an internode of about 1 cm in length. Seedlings were then transferred to 10-litre plastic containers for hydroponic cultivation in Hoagland solution.[29] Plants were kept in a growth chamber (26°C day, 18°C night, 70% RH).

Plants 20 to 30 days old, between R1 and R2 early flowering stages,[30] were used for experiments.

2.2 Pressure-chamber technique

The technique used was adapted from previous experiments.[11] Plants were decapitated just below the cotyledonary node, rinsed in calcium sulfate solution (0·5 mM) and put in a beaker inside the pressure-chamber. The whole root was bathed in water for HPLC (400 ml) containing the active ingredient at a concentration of $1{\cdot}5(\pm 0{\cdot}1)$ mg litre^{-1} (measured by HPLC using calibration with external standard).

The internode was fitted through a rubber stopper to the opening in the centre of the pressure-chamber lid. Dental impression material (Xantopren, Bayer) was used to seal the internode in the opening and the stump in a 200-μl plastic disposable pipette tip in order to avoid any damage to it. The chamber was tightly sealed. Hydrostatic pressure (0·4–0·5 MPa), generated by compressed air containing 5% oxygen, was applied to the root.

The pressure was held constant by continuously bleeding air through an exit port at the bottom of the chamber and replenishing the lost air via a device at the bottom of the chamber. The whole assembly was agitated at 70 oscillations min^{-1} and the experiments were carried out at 26($\pm$0·5)°C.

After a few minutes a constant xylem sap flow was obtained and the xylem sap was collected by a fraction collector every 15 min. The experiments were run in duplicate for most compounds.

2.3 Chemicals

Aclonifen, fenoxaprop (free acid) and imazethapyr were kindly provided by Rhone-Poulenc Agro, Hoechst and Cyanamid respectively; carbendazim, dimethoate, iprodione, linuron, metalaxyl, penconazole and simazine were purchased as analytical grade (Ehrenstorfer, Germany).

Compounds used in this study belong to different chemical families and have a wide range of lipophilicity, measured as reported in Table 1.

2.4 Acid dissociation constant (pKa)

Acid dissociation constants were determined for ionisable compounds by UV/visible spectrophotometry.[32] Briefly, different spectra of the compounds with acidic or basic functions were obtained at different pH values by adding very small quantities of hydrochloric acid (370 g $litre^{-1}$) or sodium hydroxide (320 g $litre^{-1}$) to the sample. The spectral change is due to an equilibrium between two species and the pKa was derived from the isosbestic point; values are reported in Table 1.

Acid dissociation constants were measured because certain pesticides are appreciably ionised at the pH values found in soil or plant cells and this complicates interpretation of their behaviour.[7] Root uptake and transport of weak acids is governed by the ion trap mechanism whereby the dissociated molecule is trapped in compartments of higher pH because the permeability of membranes to anions is very much lower than to neutral molecules.[16,17]

2.5 *n*-Octanol/water partition coefficient (K_{ow})

These were measured by standard procedures.[33] Measurements were by reverse-phase high-pressure liquid chromatography on octadecyl silica with methanol/water mixtures as eluent. Regression was performed between experimental *n*-octanol/water partition coefficients of 44 pesticides belonging to different chemical classes and the capacity factors measured by HPLC ($r^2 = 0{\cdot}94$).[34] Good correlation was found between the values estimated by HPLC and the experimental ones apart from metalaxyl whose log K_{ow} value was 1 unit greater than the literature value.[35]

2.6 Effects of the pressure-chamber technique on the tissue integrity and the energy metabolism status

To evaluate any potential damage to the root system created by the pressure, the physiological status of the soybean plant was checked by measuring several parameters:

(1) The concentrations of potassium in the xylem sap of detopped roots incubated with calcium sulfate (0·5 mM) and Triton X 100 (1 g $litre^{-1}$) (which damages the cellular membranes) were compared to those of

TABLE 1
Chemical Classes, pKa and log K_{ow} of the Pesticides Used

Active ingredient[a]	*Class*	*State*	*pKa*[b]	*log* K_{ow}
Aclonifen (H)	Diphenyl-ether	Non-ionic		3·98
Carbendazim (F)	Benzimidazole	Basic	4·28[c]	1·37[d]
Dimethoate (I)	Organophosphate	Non-ionic		0·77
Fenoxaprop (H)	Phenoxy	Acidic	3·13	0·003[d]
Imazethapyr (H)	Imidazolinone	Acidic	3·26	1·7[e]
Iprodione (F)	Dicarboximide	Non-ionic		3·5
Linuron (H)	Urea	Non-ionic		3·0
Metalaxyl (F)	Acylalanine	Non-ionic		1·27
Penconazole (F)	Triazole	Non-ionic		3·6
Simazine (H)	Triazine	Basic		1·93

[a] H = herbicide, F = fungicide, I = insecticide.
[b] See text for measurement procedure.
[c] Of the conjugate acid.
[d] Measured at pH = 7.
[e] From Reference 31.

roots incubated without 'Triton'. Both experiments were conducted imposing a pressure of 0·45 MPa.

K^+ concentrations were also measured in the xylem sap obtained at atmospheric pressure by exploiting root pressure,[36] which allowed collection of an appreciable volume of xylem sap (about 150 μl in 6 h).

(2) The leakage of potassium from the root cell under pressure into the incubation medium (with and without active ingredients) was compared to that of roots incubated in the same medium at atmospheric pressure.

Roots were washed in calcium sulfate (0·5 mM) which quantitatively removes potassium from the free space, before being bathed in the solution. Potassium analysis was carried out on aliquots taken just after dipping the root into the solution ($t = 0$) and at the end of the assay in the pressure-chamber.

Potassium concentrations in the xylem sap and in the external medium were determined by means of an atomic absorption spectrophotometer (Spectra AA-20, Varian).

(3) Protein levels in the xylem sap obtained from a root incubated under pressure (calcium sulfate, 0·5 mM) were compared to the levels in the xylem sap collected from a root kept in the same solution at atmospheric pressure. Protein levels were measured using a colorimetric assay (BioRad Protein Assay) by means of a UV-visible spectophotometer.[37]

(4) ATP levels in roots kept under pressure in a pesticide-free solution (calcium sulfate 0·5 mM) for 6, 12 and 14 h were compared with the levels measured in roots kept in the same solutions, continuously aerated and for the same period of time but at atmospheric pressure. ATP levels were determined in a neutralised perchloric-acid-soluble fraction of the roots by means of LKB 1243/200 ATP- monitoring reagent in an LKB-Wallac 1250 Luminometer.[38]

2.7 Analysis of pesticide in xylem sap

Xylem sap was injected directly into the HPLC Varian 9010, equipped with a Varian 9065 diode-array detector and a stainless steel cartridge column (15 cm × 4·6 mm ID) packed with Spherisorb ODS (5 μm). The injection loop was 100 μl; flow rate was 1 ml min^{-1}; wavelength and eluent mixtures were chosen according to the best chromatographic performance of each active ingredient (Table 2). Concentrations of solution aliquots containing each active ingredient were measured using calibration with external standard.

2.8 Statistical analysis

A *t*-test for K^+ leakage, analysis of variance for the ATP contents and non-linear regression analysis[39] for interpolation of pesticide concentration in xylem sap were performed using SAS software (SAS release 6.08).

3 RESULTS AND DISCUSSION

3.1 Effects of hydrostatic pressure on soybean plants

Potassium leakage is a parameter often used to determine potential damage to cell membranes. If the pressure of 0·4–0·5 MPa causes damage to the cell membrane, then K^+ may leak from the cell into the xylem sap as well as into the external medium. For the same reasons, protein levels in the xylem sap were also measured.

ATP levels in the cell root were measured to assess whether the oxygen availability in the pressure-chamber was sufficient for the metabolic functions.

TABLE 2
Retention Time, Wavelength of Maximum Absorption and Analytical Conditions Used

Active ingredient	*RT* (*min*)	λ_{max} (*nm*)	λ_{used} (*nm*)	H_2O (%)	CH_3CN (%)	CH_3OH (%)
Aclonifen	8·17	195	314	30	—	70
Carbendazim	5·5	205	283	[a]		
Dimethoate	3·3	190	210	80	20	—
Fenoxaprop	8·4	195	240	30[b]	—	70
Imazethapyr	3·8	199	254	65[b]	35	—
Iprodione	3·3	205	210	30	70	—
Linuron	3·0	210	210	30	70	—
Metalaxyl	3·2	195	210	30[c]	—	70
Penconazole	2·3	195	210	[d]		—
Simazine	2·64	220	220	30[b]	—	70

[a] Gradient: CH_3CN/buffer KH_2PO_4-NaOH 0·002 M pH 7 from 15/85 to 40/60 in 20 minutes.
[b] Buffer NaH_2PO_4/H_3PO_4 0·01 M pH 2·5.
[c] Buffer NaH_2PO_4/H_3PO_4 0·01 M pH 7.
[d] Gradient: H_2O/CH_3CN from 80/20 to 0/100 in 40 minutes, column Spherisorb ODS 2·5 μm, 15 cm, ID 4·6.

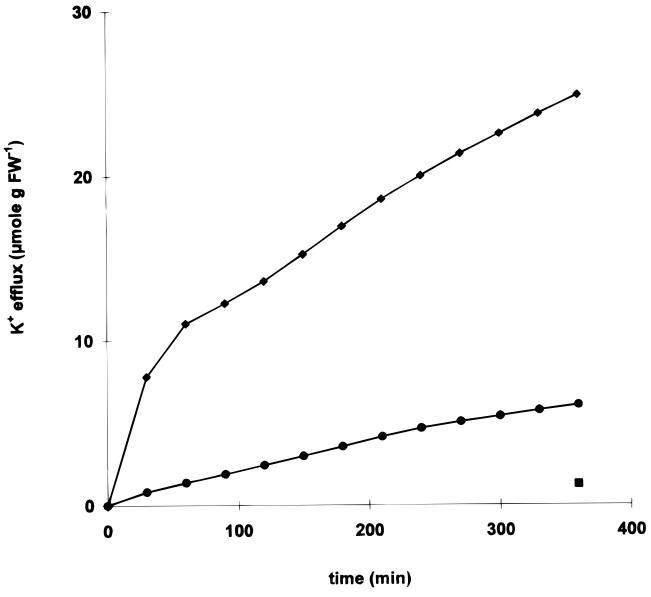

Fig. 1. K^+ leakage in xylem sap, (◆) in presence of 'Triton' X-100 at 0·4 MPa, (●) control at 0·4 MPa, (■) at atmospheric pressure.

3.1.1 Potassium concentrations in the xylem sap

The K^+ concentrations in the xylem sap of roots incubated with 'Triton' and exposed to 0·45 MPa of pressure were remarkably greater those in the xylem sap of roots incubated in the same conditions without 'Triton' (control). In the absence of 'Triton', application of the external pressure induced a slightly greater release of K^+ into the xylem sap than was obtained at atmospheric pressure (Fig. 1).

3.1.2 Potassium leakage into the external medium

The amount of K^+ released into the incubation medium by roots under pressure was not statistically different from the amount released by roots incubated at atmospheric pressure ($P > T = 0{\cdot}64$, $df = 10$) (Table 3). Moreover the pesticides, over the time and the concentration used, did not consistently affect the K^+ leakage from roots, indicating that they caused no severe damage to membrane integrity and functionality (Table 3).

3.1.3 Protein leakage into the xylem sap

Very low protein levels were determined in the xylem sap. The levels were similar to those measured in the xylem sap collected at atmospheric pressure (data not shown).

3.1.4 ATP content of roots

The ATP concentrations measured in roots kept under pressure in pesticide-free solutions for 6, 12 and 14 h were not statistically different ($F = 0{\cdot}91$, $P > F = 0{\cdot}36$, $df = 11$) from levels measured in roots kept at atmospheric pressure (Table 4).

The oxygen supply in the pressure-chamber experiments seems to satisfy the requirements for metabolic functions.

The potassium leakage, both into the xylem sap and into the external medium, the absence of detectable protein in the xylem sap and the ATP contents seem to support the hypothesis that the pressure-chamber technique does not cause damage to the soybean roots. Also, the hydrostatic pressure used was less than the value of the water potential measured in xylem of roots near the ground surface (0·6 MPa).[36]

The sap flow obtained in detopped soybean plants (average 25 g intact plant FW) of $4{\cdot}4(\pm 0{\cdot}76)$ ml h^{-1} g FW^{-1} was comparable to the transpiration rate of 10–13 ml h^{-1} $plant^{-1}$ measured in a growth chamber with 30-day-old soybean plants (average 53 g FW).[40] Again, these data indicate that the pressure used was not very different from the tensions arising under natural conditions.

TABLE 3
Potassium Leakage into the External Medium[a]

Pesticide	*Trial duration (min)*	*K^+ leakage (µmol g FW^{-1})*	
		Pressure-chamber	*Control*
None	600	4·8	3·5
Aclonifen	600	5·93	6·85
Carbendazim	600	5·85	6·20
Dimethoate	420	3·19	4·10
Fenoxaprop	840	5·61	6·15
Imazethapyr	360	5·50	5·54
Iprodione	375	6·96	5·54
Linuron	390	2·40	3·63
Metalaxyl	600	8·72	5·40
Penconazole	450	3·82	4·37
Simazine	300	9·54	4·50

[a] Results are the difference between K^+ concentrations measured at the end and at the beginning of the experiment ($t = 0$).

TABLE 4
ATP Concentrations in the Root Tissue

Time (*min*)	*ATP levels* ($\pm SD$)[a] (*nmol ATP g* FW^{-1})	
	Pressure-chamber	*Control*
0	24·1 (±1·3)[b]	26·5 (±1·5)[b]
6	40·2 (±2)	37·1 (±1·5)
12	33·7 (±1·4)	26·8 (±1·5)
14	24·1 (±1·3)	27·7 (±2·6)

[a] n = 3.
[b] ATP measured in excised and immediately frozen roots.

3.2 Efficiency of translocation

The efflux curves of all tested compounds into root xylem sap, apart from imazethapyr, showed a steady-state kinetic profile (Figs 2a and 2b) analogous to those obtained in previous experiments in intact root systems[11,21] and in a stem perfusion experiment.[22] The different behaviour of imazethapyr is probably due to its rapid metabolisation in soybean plants (data not shown). The time required to obtain a steady-state concentration and the steady-state concentration differed for the different compounds. The time required to achieve 50% of the steady-state concentration ($TSSC_{50}$) and the steady-state concentration were calculated for each chemical, interpolating the efflux data by non-linear regression analysis.[39] The best models obtained were: sigmoid (fenoxaprop, iprodione and penconazole), exponential saturation (aclonifen, carbendazim (MBC) and simazine) or rectangular hyperbola (dimethoate, linuron and metalaxyl).

Dimethoate, the least lipophilic non-ionised pesticide tested, reached the steady-state concentration before any other compound, whereas aclonifen, the most lipophilic one, required the longest time. The kinetic profile of aclonifen in the xylem sap is quite similar to that obtained with a lipophilic analogue[11] but, since fractions of xylem sap were combined in 4-h samples for analysis, the authors were not able to measure the concentration of the compound at the beginning of the experiments.

The $TSSC_{50}$ value is positively correlated with the lipophilicity of the molecule: the more lipophilic the compound, the more time was required to reach a steady-state concentration (Fig. 3); dimethoate and aclonifen, the least and the most lipophilic compounds, respectively, showed the lowest and the highest $TSSC_{50}$ values. This correlation may be explained on the basis that a chemical with a relatively high lipophilicity increases its partition in all the lipophilic cell structure because it may easily cross the plasma membrane of root hairs, cortex and stele cells and consequently its

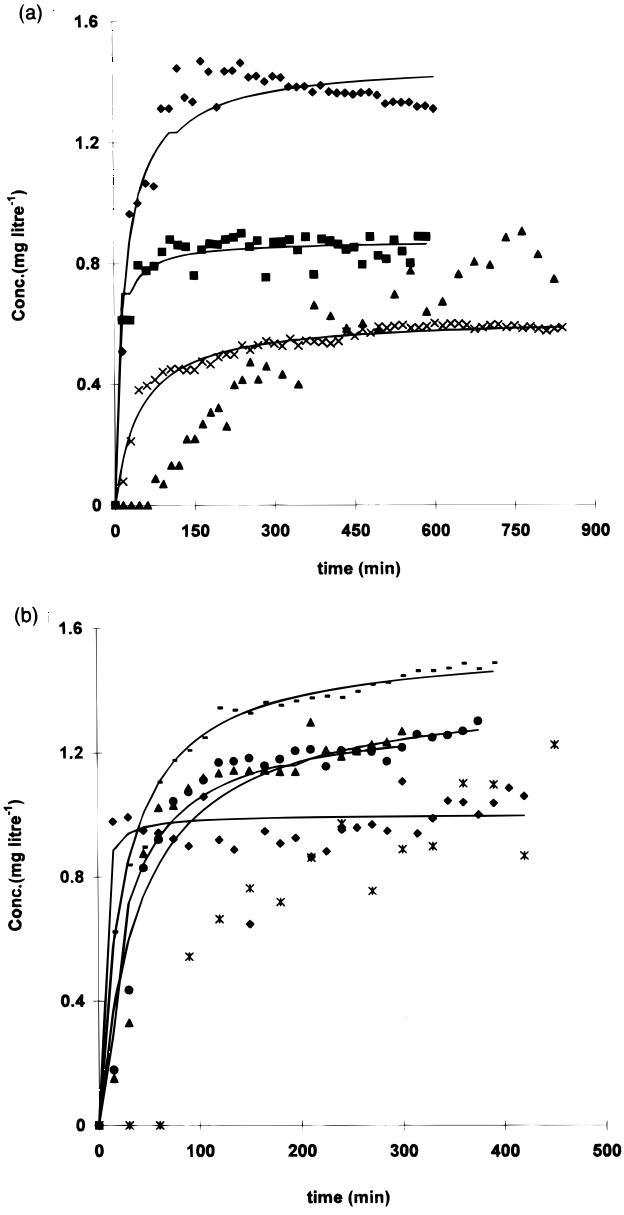

Fig. 2. Pesticides in xylem sap. (a) (◆) MBC, (■) metalaxyl, (▲) aclonifen, (×) fenoxaprop. (b) (◆) dimethoate, (▲) simazine, (★) penconazole, (●) iprodione, (−) linuron.

movement in the root is symplastic. The high affinity for the lipophilic structure of the cell in turn affects the time required to reach the steady-state concentration into the xylem sap, achieved only when all sites of interaction with lipidic phases of the cells are saturated.

A chemical of low lipophilicity is less available to move from external solution to stele by a symplastic pathway and is less partioned than a more lipophilic one into the lipid cell structure. Compounds of low lipophilicity move mainly by water mass flow through the root apoplast, up to the Casparian strip, reaching the steady-state concentration into the xylem sap more quickly. This interpretation is in agreement with the results obtained in previous experiments.[14] The shape of the curve where the root concentration factor was

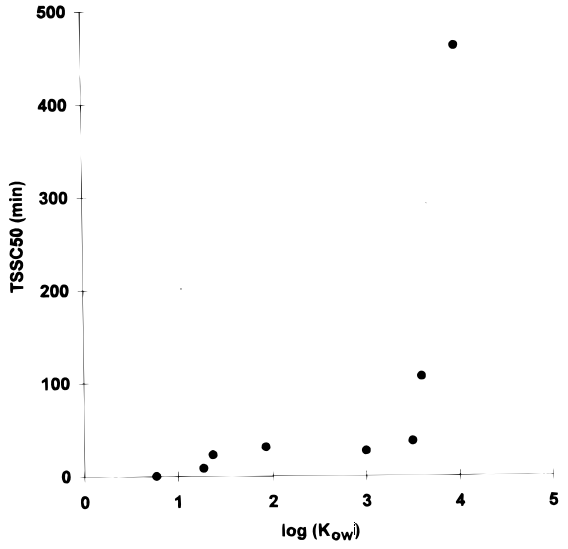

Fig. 3. $TSSC_{50}$ as a function of lipophilicity for non-ionised compounds.

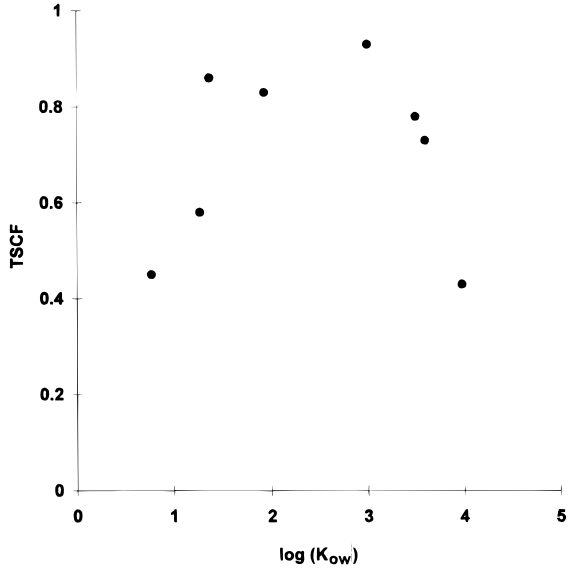

Fig. 4. TSCF as a function of lipophilicity for non-ionised compounds.

plotted as a function of log K_{ow} established by Briggs *et al.*[14] is in fact similar to the shape of the curve where the $TSSC_{50}$ is plotted as a function of log K_{ow} (Fig. 3).

The efficiency of movement of a molecule into the shoot from the roots is usually expressed as the transpiration stream concentration factor (TSCF).[4,5,14] At the end of each experiment, the concentration of compound remaining in the solution in the pressure-chamber was determined. The value of steady-state concentration for each compound was then used for the calculation of TSCF according to Hsu *et al.*:[11]

$$\text{TSCF} = \frac{\text{Concentration in xylem at steady-state efflux}}{\text{concentration in external solution at end of experiment}}$$

The relationship between TSCF and log K_{ow} is reported in Fig. 4. The efficiency of translocation was best achieved by those compounds with log K_{ow} values ranging from 2 to 3, decreasing for compounds of lower as well as higher lipophilicity. This trend is similar to that found by several authors and expressed as parabolic[41] or Gaussian[11,14] relationships.

The steady-state concentration reached by a pesticide into the xylem sap depends on its permeability to plasmalemma, at least to get past the Casparian strip, since it must cross the endodermis to reach the xylem vessels. This permeability is positively correlated with the lipophilicity of the compound, with maximum permeation occuring at a log K_{ow} value of approximately 3.

The TSCF is also reduced above this optimal log K_{ow} value because of the increasing partition into the lipophilic cell structure displayed by more lipophilic pesticides, a phenomenon that curtails the amount of the compound available to the xylem sap.

On this basis the best TSCF values are achieved for the chemicals characterised by an optimal log K_{ow} value representing the best integration between two contradictory factors, partition into xylem sap and permeability to plasma membranes. On the one hand, pesticides of low lipophilicity partition into xylem sap very well but their efficiency of translocation is curtailed because their permeability to membranes is less than optimal, and this phenomenon diminishes the ease with which such molecules cross the endodermis.

On the other hand, pesticides of high lipophilicity that may permeate the plasma membrane easily are scarcely partitioned into xylem sap as a consequence of their high affinity for the lipidic cell structure.

Binding may also occur onto other non-lipidic sites of the root structure (e.g. cell wall) with high affinity for the pesticide, this being possible for chemicals with very low log K_{ow} values.

The TSCF optimum occurred at a log K_{ow} value of 3, similar to that measured using the pressure-chamber technique,[11] where values are higher than those measured directly in shoots for two series of nonionised compounds.[14] Although there is a general similarity of plant membranes in different species,[18] contrasting behaviour of barley plants in comparison with soybean plants in uptake experiments has been found.[5] Further investigations are required to determine if this difference is associated with the contrasting morphology of the root systems of the two species or imposed by the experimental method which exploits the pressure-chamber technique.

4 CONCLUSIONS

Experiments reported in the present study suggest that the pressure-chamber technique is useful for studying

the xylem translocation of pesticides. The physiological status of the soybean plant used under pressure showed that this technique does not damage either the tissue integrity or the energetic metabolic state of the root. The general translocation pattern into the xylem sap of pesticides belonging to several chemical classes appears to be largely determined by the lipophilicity of the compound. The time required to achieve 50% steady-state concentration depends on the lipophilicity of the pesticide. The more lipophilic a compound, the more time is required to reach the steady-state concentration. The results suggest that the soybean plants translocate different pesticides to different extents. The highest translocation is achieved with compounds of intermediate lipophilicity.

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